TOTAL COLIFORM, E. COLI, AND ENTEROCOCCI BACTERIA IN GRAZED AND WOODED WATERSHEDS OF THE SOUTHERN PIEDMONT

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Abstract. Contamination of surface waters with fecal bacteria from grazinglands is a component of non-point source agricultural pollution. Methods are needed to limit the movement of fecal bacteria from grazinglands into surface water. We used two experimental watersheds to test for impact of cattle on total coliform, *E. coli*, and enterococci bacteria numbers. Grazing cattle elevated these microbe numbers but we found that positioning animals above a pond in the landscape was an effective means of reducing total coliform, *E. coli*, and enterococci bacteria in surface water leaving the grazed watershed. Microbe numbers in the pond outflow were similar to those in surface water from wooded watershed.

INTRODUCTION

Urban development and the consequent increase in demand for surface water to supply domestic and recreational needs means that movement of pollutants from agricultural lands must be controlled (Nader et al., 1998). Fecal microbe numbers in water from grazinglands can be quite high (Jawson et al., 1982) and yet the relative lack of field studies has been noted by researchers attempting to model agricultural impacts compared with natural sites (Fraser et al., 1998). Exclusion of cattle from surface water by fencing is partially effective but expensive and runoff from pastures during periods of high rainfall may still contaminate streams. Additional economically viable methods of reducing microbe numbers are needed for beef production systems in the southeastern USA.

Historically, tests for total or fecal coliform bacteria have been used to indicate fecal contamination but these tests enumerate some microbes that are not necessarily fecal in origin and can yield high estimates from natural areas due to wildlife. Tests that are specific for *E. coli* and enterococci may be more useful for detecting the impact of grazing animals but have not been tested for that purpose.

The objectives of this work were first to compare numbers of total coliform, *E. coli*, and enterococci bacteria in the surface water of a watershed grazed by cattle with surface water of a wooded watershed. Secondly, our objective was to test the impact of a retention pond on the numbers of microbes in water leaving a grazed watershed.

METHODS

Two watersheds with contrasting land uses were identified on the J. Phil Campbell, Sr., Natural Resource Conservation Center near Watkinsville, GA. One watershed was used primarily for grazing and portions of the riparian areas were accessible to cattle during portions of the grazing season. The other watershed was wooded and no domesticated ruminants were located within approximately 1 km of the site. Both watersheds had springs at the head of perennial creeks and the grazed watershed had an approximately 2-ha pond on the creek flowing from the pastures.

Within both watersheds the springs and creeks were selected as sampling sites (Table 1). In addition, within the grazed watershed, the pond and the outflow from the pond was sampled. The pond was sampled from the

Table 1. List of Sampling Sites with Approximate Contributing Hectares Based on Topography.

Site	Contributing Area
	-- ha --
Grazingland Spring	8
Grazingland Creek	47
Grazingland Pond	98
Grazingland Pond Outflow	98
Wooded Spring	14
Wooded Creek	38

Table 2. Mean Total Coliform and E. Coli Bacteria per 100 ml (Values Followed by the Same Letter are not Significantly Different, n=41).

Site	Total Coliforms	E. coli
	------ MPN/100ml ------	
Grazingland Spring	543 c	2 e
Grazingland Creek	7121 a	894 a
Grazingland Pond	3963 b	19 c
Grazingland Pond Outflow	3525 b	17 c
Wooded Spring	576 c	8 d
Wooded Creek	3162 b	88 b

surface near the bank approximately 1/3 of the distance between the point at which the creek entered the pond and the dam. The sample that flows out of the pond was collected near the base of the dam from a pipe that collects overflow and releases it below the pond.

Two grab samples of 100 ml were collected at each site at approximately 1-week intervals for a year (n=41). One sample was tested for total coliform and *E. coli* simultaneously and the other sample was tested for enterococci bacteria using Colilert® and Enterolert™ substrates for tests that are specific to those organisms (IDEXX Laboratories, Inc., Westbrook, ME). Samples were enumerated by presence and absence in Quanti-Tray™ cells (IDEXX Laboratories, Inc., Westbrook, ME) and data were expressed as the most probable number (MPN) per 100 ml. All sites were sampled on each sample date. To avoid saturating the assays, samples were diluted at ratios of 1 to 10 or 1 to 100 when we anticipated high numbers of microbes. However, assays occasionally saturated at MPNs of 2419.17, 24191.7, or 241917 MPN/100ml depending on dilution rate.

Preliminary data analysis indicated the need for a transformation to reduce skewness and kurtosis in all three variables. A log transformation was effective and analysis of variance was conducted on the transformed data. The log of 1 is zero and the log of zero is not defined. Therefore observations of zero were set to zero in the transformed data set. Skewness ranged from 5 to 8 in the original units and was reduced to less than 1 by transformation. Kurtosis ranged from 23 to 82 in the original units and was reduced to less than 1 by transformation.

Ratios between microbial assays have been explored as a means of differentiating between contamination by cattle and contamination by wildlife (Baxter-Potter and Gilliland, 1988). Consequently we calculated the ratio of *E. coli* to total coliforms and the ratio of enterococci to total coliforms. The numbers of *E. coli* and enterococci were used in the numerator to avoid placing zero observations in the denominator and to maintain a balanced data set. These variables also required a log transformation prior to calculation of the ratio and analysis of variance. Skewness ranged from 3 to 10 in the original units and was reduced to less than 1 by the transformation. Kurtosis ranged from 10 to 125 in the original units and was reduced to from –1.3 to 0.4 by the transformation.

All variables were tested by analysis of variance with site (n=6) and date (n=41) as class variables and as the only components in the model using the GLM procedure of SAS (SAS, 1990). A Waller-Duncan t-test (k ratio=100) was used to separate the means. Letters were assigned indicating significant differences and the values transformed into the original units for presentation. Simple linear correlation (r) was also calculated to examine the relationship between the three microbial assays.

RESULTS AND DISCUSSION

Total Coliform Bacteria

Water from the two springs had similar numbers of total coliform bacteria (Table 2). The coliform analysis is not specific to bacteria of fecal origin and may be related to decaying organic matter surrounding the springs. The impact of the grazing cattle was evident in the elevated coliform numbers in the grazingland creek. However, the number of coliforms in the wooded creek was also elevated relative to the wooded spring. Wildlife activity in the wooded watershed might have contributed to the concentrations of total coliforms in the surface water. In the grazed watershed, total coliforms were lower in the pond and in the outflow from the pond than in the creek that flowed into the pond. Total coliform numbers may have been reduced while in the pond due to sedimentation and/or decay.

E. Coli Bacteria

Levels of *E. coli* were 2 to 50 times lower than total coliform bacteria (Table 2). The test for *E. coli* is a more specific indicator of fecal contamination and there was a large increase in *E. coli* numbers in the grazinglands creek compared with all other sites. Numbers of *E. coli* in the grazinglands creek were nearly 400 times those in the spring and were 10 times the number in the wooded creek. Low numbers of *E.*

Table 3. Enterococci Bacteria per 100 ml (Values Followed by the Same Letter are not Significantly Different, n=41).

Site	Enterococci
	--- MPN/100ml ---
Grazingland Spring	2 e
Grazingland Creek	174 a
Grazingland Pond	6 c
Grazingland Pond Outflow	5 dc
Wooded Spring	3 d
Wooded Creek	10 b

Table 4. Ratios of E. Coli and Enterococci to Total Coliform Bacteria (Values Followed by the Same Letter are not Significantly Different, n=41).

Site	E. Coli to Total Coliforms	Enterococci to Total Coliforms
Grazingland Spring	0.10 d	0.06 d
Grazingland Creek	0.77 a	0.58 a
Grazingland Pond	0.38 c	0.22 bc
Grazingland Pond Outflow	0.37 c	0.22 bc
Wooded Spring	0.29 c	0.17 c
Wooded Creek	0.57 b	0.29 b

coli were found in the springs. The pond was very effective in reducing *E. coli* numbers. Numbers of *E. coli* in the pond outflow were even lower than the numbers of *E. coli* found in the creek of the wooded watershed.

Enterococci Bacteria

The enterococci assay is also a more specific test than total coliforms for fecal contamination. Enterococci numbers were lower than either *E. coli* or total coliform bacteria (Table 3). Presence of cattle elevated the numbers in the grazingland creek to approximately 17 times the number in the wooded creek. Both springs were low in enterococci numbers and the pond was effective in reducing numbers prior to discharge into the creek and out of the grazinglands.

Correlations among Microbial Assays

Each microbial assay was significantly correlated with the other two assays ($n = 246$, $p<0.01$) but correlations were not large. The correlation (r) with total coliforms was only 0.47 for *E. coli* and 0.45 for enterococci. The correlation between *E. coli* and enterococci was 0.67. However, a correlation of 0.67 indicates that only approximately 45% of the variation observed in one variable could be explained by the other. The assays may be most valuable as a suite of measurements used to estimate fecal contamination.

Range of Microbe Numbers

All three assays saturated on some sample dates in spite of efforts to dilute samples suspected of having particularly high numbers of microbes. This occurred with approximately 13% of the total coliform samples, 1% of E. coli samples, and 2% of enterococci samples. Although the data were log-normally distributed and large numbers were rare, each site was occasionally much higher than expected. The means reported are the antilogs of the means of transformed numbers but the range observed in the data set is also of interest. Even the spring in the wooded watershed had total coliforms has high as 241920 MPN/100ml, *E. coli* as high as 17200 MPN/100ml, and enterococci as high as 387 MPN/100ml. The narrowest range was observed at the spring in the grazed watershed. Total coliforms ranged from 138 to 10462 MPN/100ml, *E. coli* from 0 to 142 MPN/100ml, and enterococci from 0 to 200 MPN/100ml. At the grazinglands spring, samples were collected from the flow out of a plastic pipe that had been set in the mouth of the spring so sample bottles could be filled with less chance of contamination by wildlife. This was not possible at the wooded spring and we collected samples from a pool formed by the spring. We suspect that on some sample dates wildlife had contaminated the spring in the wooded watershed. The flow of both springs was estimated to be a few liters a minute and the spring would eventually flush contamination down stream. However, some samples from the wooded spring may have been affected by recent fecal contamination by wildlife.

Ratios of Microbe Numbers

Ratios reflected the elevated *E. coli* and enterococci at the grazingland creek (Table 4). The grazingland spring had the lowest ratio and the pond samples were similar to the wooded spring. The two creeks were differentiated by this method but further work would be required to determine if this procedure adds any substantive information over simply enumerating *E. coli*, enterococci, and total coliforms. The coefficients of variation (CV) were not improved with the ratio. The CV from the analysis of variance of the log transformed *E. coli* was 56% and from the *E. coli* to total coliform ratio it was 55%. In the case of

enterococci, analysis of variance of both the ratio and the microbe numbers resulted in CVs of 70%. The ratios did not differentiate between the wooded spring and the grazingland pond and in this case did not add to our ability to discern between the grazed wooded watersheds.

SUMMARY

Assays for *E. coli* and enterococci were more sensitive to the presence of cattle in the watershed than total coliforms. Total coliforms were about 2.25 times as numerous in the grazinglands creek as in the wooded creek. In contrast, *E. coli* numbers were 118 times and enterococci were 17 times as high in the grazingland creek as they were in the wooded creek. Consequently, either an assay for *E. coli* or enterococci would be more likely to differentiate the impact of cattle from wildlife than an analysis for total coliforms. The high numbers of bacteria enumerated by the total coliform procedure in both natural and grazed areas complicates discernment of agricultural impacts.

The relatively low correlation between the three assays warrants further investigation. A combination of the assays may be most effective for detecting fecal contamination that is not simply a result of wildlife. However, simply calculating a ratio of *E. coli* or enterococci to total coliforms was not as effective as simply using *E. coli* or enterococci numbers.

Locating grazing animals in the landscape above a pond is an effective means of reducing numbers of total coliforms, *E. coli*, and enterococci bacteria in surface waters leaving grazinglands. Runoff events are most common in the winter months during periods when cattle may be concentrated and fed supplements. It might be possible to position these pastures higher in the landscape so that runoff will flow to a pond. Further research is needed to determine the size requirement for the pond relative to the contributing land area. However, pond placement appears to be an economic and promising procedure for improving water quality flowing from grazinglands.

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